Tissue and Cell 50 (2018) 133–143

Contents lists available at ScienceDirect

Tissue and Cell

journal homepage: www.elsevier.com/locate/tice

Original paper

Protective roles of Rutin against restraint stress on spermatogenesis in testes of adult mice

Asif Mehfooz, Quanwei Wei, Kaizhi Zheng, Mohamed Babo Fadlalla, Gracijela Maltasic, Fangxiong Shi*

College of Animal Science and Technology, Nanjing Agricultural University, Nanjing, 210095, China

ARTICLE INFO

Keywords:
Restraint stress
Rutin
Testis
PARP1

ABSTRACT

In order to investigate the effects of Rutin against restraint stress, 50 adult male mice were divided into five groups: control, restraint stress (RS), and RS with 2 doses of Rutin treatments. Mice were restrained in a conical tube for 4 h daily and Rutin was injected intraperitoneally for 15 consecutive days. Restraint stress significantly decreases body weights, testis and epididymis weights, thymus weights, visceral fats, serum concentrations of testosterone, sperm counts, sperm motility and sperm viability, while it increases serum epinephrine levels, adrenal gland weights and abnormal sperms. In addition, restraint stress severely damages the testicular histoarchitecture and spermatogenesis. Stressed groups also showed broken seminiferous tubules, few spermatozoa in lumen, less population of Leydig cells between the interstitial spaces, spermiation arrest in stage I–III and degenerated population of round spermatids in the lumen; as well as missing cells in stages IV–VI. Furthermore, lumen sizes increased in stages VII, VIII, IX and X. Residual bodies increased in stages IV–VI, VII–VIII and vacuoles found in stages XI–XII after restraint stress. PARP1 signaling is involved in apoptosis. In this study, expressional levels of cleaved PARP1 and cleaved Caspase-3 are significantly increased in testes after restraint stress. We demonstrate that Rutin significantly ameliorates the side effects induced by restraint stress.

1. Introduction

Living organisms survive by maintaining complex dynamic and harmonious equilibrium. This equilibrium is constantly challenged or outright threatened by intrinsic or extrinsic stressors. Stress is a state of disharmony or threatened homeostasis by stressors (Chrousos and Gold, 1992). Generally, there are three types of stressors, which are physical (e.g., restraint, foot shock, and exercise), psychological (including isolation, anxiety, fear, or mental frustration) and metabolic (including upright tilt, heat exposure, hypoglycemia, and hemorrhage) (Weissman et al., 2007). Restraint (immobilization) stress is an amalgamation of both physical and psychological stress, whereby movement is confined to a restricted area and the individual is isolated from its group (Pacak and Palkovits, 2001). It is an experimental procedure developed for biomedical studies of stress (Glavin et al., 1994). Restraint stress impedes male reproductive capacity by hampering the hypothalamic-pituitary-testicular axis (Rai et al., 2004). Previous studies have reported that acute immobilization and chronic intermittent immobilization stress increase circulating glucocorticoid concentrations, while it decrease gonadotropin-releasing hormone (GnRH), follicle stimulating hormone (FSH), luteinizing hormone (LH) and testosterone concentrations, resulting in Leydig cell and seminiferous tubule damages and germ cell apoptosis in adult rat testes (Arun et al., 2016a; Everds et al., 2013).

Our previous studies have revealed that restraint stress results in impaired growth performance and testicular cell apoptosis in male mice (Mehfooz et al., 2017). Such damage in testes could somehow be prevented by Big-leaf mulberry in rats subjected to water immersion and restraint stress (Xu et al., 2014); the IGF-1/PTEN/Akt/FoxO signaling pathway involved in the testis and stomach of rats following water immersion and restraint stress (Huang et al., 2012a,b). However, the effective measures to reduce this damage are lacked.

Rutin is a plant pigment (flavonoid) found in buckwheat, passion flower, apple and green tea. It has been reported to exhibit various pharmacological properties including antioxidant, anticarcinogenic, cytoprotective, antiplatelet, antithrombotic, vasoprotective, anti-inflammatory and cardioprotective effects (Salem et al., 2017). The strong antioxidative capacity of Rutin has been proven by numerous studies, particularly for excellent scavenging activity (Duthie and Dobson, 1999; Leong et al., 2008). However, mechanisms of Rutin against stress still need clarifying.

Apoptosis is a programmed process of cell death and the tissue in

* Corresponding author.
E-mail addresses: weiquanwei@njau.edu.cn (Q. Wei), fxshi@njau.edu.cn (F. Shi).

https://doi.org/10.1016/j.tice.2018.01.003
Received 30 July 2017; Received in revised form 20 December 2017; Accepted 9 January 2018
Available online 10 January 2018
0040-8166/

which a high incidence of apoptosis occurs in a vertebrate's body is the testis, whereby 75% of all male germ cells produced are discarded through the process of apoptosis (Shaha et al., 2010). Poly (ADP-ribose) polymerase-1 (PARP-1) is involved in apoptosis and necrosis (Brugnon et al., 2009). Caspase-3 inactivates PARP1 by cleaving PARP1 into two parts, which causes cell apoptosis. Exogenous agents such as reactive oxygen species (ROS) or genotoxin may cause DNA damage in a cell. Our previous studies have revealed that PARP-1 cleavage was implicated in female pig reproduction (Wei et al., 2013, Wei and Shi, 2013). However, its role in male reproduction is lacked.

The aim of our present study is to evaluate the protective effects of Rutin against restraint stress on endocrine, histoarchitecture and histopathology, caspase-dependent pathway of apoptosis, sperm morphology and spermatogenesis in testes of adult mice.

2. Materials and methods

2.1. Animals

Young adult male Swiss ICR (Institute for Cancer Research) mice were purchased from Qinglongshan Laboratory Animal Company (Nanjing, China) at 60 days old. They were kept under control environment consisting of a 12-h light: 12-h dark cycle, humidity at 60–70% and a room temperature of 22–23 °C. Mice were fed with standard balanced rodent pellets and drinking water was made available *ad libitum*. Animals were handled to become adapted for at least 7 days prior to the beginning of the experiment. The experimental protocols involving mice were approved in accordance with the Guide for the Care and Use of Laboratory Animals prepared by the Institutional Animal Care and Use Committee of Nanjing Agricultural University [Authorization number for experimental animals (SYXY (SU) 2015-0015)].

2.2. Experimental design

Fifty adult male mice were used in this study. The animals were divided into five groups with 10 mice in each group; mice were subjected to treatment and others without Rutin treatments. Rutin (Sigma Chemical Co., St Louis, MO, USA) was dissolved in 10 µl of vehicle (DMSO: Dimethyl Sulfoxide) and a dose of 20 mg/kg and 200 mg/kg (Nassiri-Asl et al., 2013) was injected intraperitoneally for 15 days consecutively. These doses were calculated according to the average body weights of each group. The control group mice weren't exposed to restraint stress (RS) and treatments of Rutin, RS group were mice restrained in a conical tube for 4 h daily for 15 consecutive days, RS-V group were mice restrained in a conical tube for 4 h daily and injected with 10 µl only vehicle (DMSO) intraperitoneally for 15 consecutive days, RS–20 mg and RS–200 mg groups were mice restrained in a conical tube for 4 h daily and injected with 10 µl Rutin of 20 mg/kg and 200 mg/kg intraperitoneally for 15 consecutive days, respectively (Fig. 1).

Mice were sacrificed under CO2 anesthesia after 15 days, and blood samples were collected and centrifuged at 4000 rpm for 10 min to retrieve sera and then stored at −80 °C until use. Testes, epididymis, adrenal glands, thymus and visceral fats were collected and weighed, and portions were stored at −80 °C, while others were fixed in 4% paraformaldehyde (PFA) for histological and immunohistochemical examination.

2.3. Restraint stress protocol

According to the reported methods (Iwakabe et al., 1998), mice were physically restrained in a 50 ml conical centrifuge tube in which various holes for ventilation with a diameter of 0.4 cm had been drilled. Each individual mouse was restrained in a 50 ml tube for 4 h (h) daily consecutively for 15 days without supplying food and water (Mehfooz et al., 2017). Control mice were left in usual animal cages for the same time without food and water.

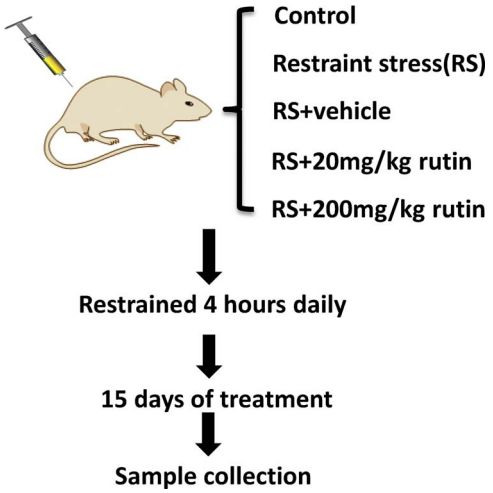

Fig. 1. Schematic illustration of the experimental setup. Mice were injected with Rutin which was dissolved in 10 µl of DMSO and a dose of 20 mg/kg or 200 mg/kg was injected intraperitoneally for 15 days consecutively. These doses were calculated according to the average body weights of each group.

2.4. Measurement of body, testis, epididymis, adrenal gland, thymus and abdominal fat tissue weights

To assess whether restraint stress affects the total weights of testes, epididymis, adrenal glands, thymus and abdominal fat tissue, the weight of these tissues were measured. There are two major compartments of abdominal fat in mouse: subcutaneous adipose tissue (SAT) is present between the skin and the abdominal wall, and visceral adipose tissue (VAT) which surrounds the abdominal organs. We collected the SAT and VAT surrounded kidneys, intestine and testes in our present study.

2.5. Measurement of feed index [grams(g) per 10 g] and water index [milliliters(ml) per 10 g]

Feed index (g/g) and water index (ml/g) were calculated for each group as follows: food consumption index = total food consumed per day/body weight × 10; water intake index = total water intake per day/body weight × 10. The method for this measurement is according to our published papers(Korejo et al., 2016; Mehfooz et al., 2017).

2.6. Radioimmunoassay (RIA) for serum concentrations of EPI (epinephrine) and testosterone

Blood was collected from mice of different groups and centrifuged at 5000 × *g* for 10 min to separate the serum. Then it was used to determine epinephrine (Beijing North Institute of Biological Technology, Beijing, China, No. KYA02) and testosterone (Beijing North Institute of Biological Technology, Beijing, China, No. B10TFB) by commercial RIA kit, which is performed in the General Hospital of the Nanjing Military Command, China. The intra-assay and inter-assay coefficients of variation for testosterone were < 10% and < 15% respectively. The intra-assay and inter-assay coefficients of variation for epinephrine were less than 9% and 15% respectively. The protocol for RIA was according to our previous study(Fadlalla et al., 2017) and published literature (Dorgan et al., 2002).

2.7. Sperm analysis

Sperm from adult mice were isolated as described previously